

Original Paper

# Kinetics of Hepatitis B Virus Load During Haemodialysis Sessions and $\alpha$ -Interferon: A Prospective Study

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## Key Words

Hepatitis B Virus (HBV) • Hemodialysis • HBV viremia • HB surface antigen (HBsAg)

## Abstract

**Background:** It has been reported a slow progression of hepatitis B in patients undergoing maintenance dialysis, and a role of dialysis session *per se* has been suggested. The aim of the present study is to evaluate the kinetics of the hepatitis B viral load (HBV DNA) in serum during haemodialysis sessions using a highly sensitive technique; the role of interferon- $\alpha$  in lowering HBV viral load in such patients was also investigated. **Methods:** HBV DNA was determined in 24 HBsAg positive patients on maintenance hemodialysis immediately before and after a 4-hour hemodialysis session, the same measurements were repeated 48 and 72 hours later. HBV DNA quantitation was performed by a novel RealTime PCR assay. Serum IFN- $\alpha$  levels were tested in parallel in a subset of HD sessions ( $n=40$ ) by ELISA. **Results:** 20 (83%) HBsAg positive patients had detectable HBV DNA in serum. Positive status for HBV DNA in serum was not predicted by demographic, clinical or biochemical parameters. HBV load decreased in many patients after hemodialysis sessions,  $5.92 \log_{10}$  IU/mL (95% CI, 5.34 to  $6.28 \log_{10}$  IU/mL) vs.  $4.79 \log_{10}$  IU/mL (95% CI, 4.23 to  $6.15 \log_{10}$  IU/mL) ( $P=0.02$ ). A significant relationship between mean HBV DNA levels before dialysis and percentage reduction of HBV DNA during HD sessions occurred [ $F$ -test=5.41,  $\rho$  (least squares)=0.307]. Increase of serum IFN- $\alpha$  levels was found in a minority (3/40=7%) of HD sessions. **Conclusions:** Hemodialysis procedure gives reduction of HBV load in HBsAg chronic carriers; no relationship with IFN- $\alpha$  activity during HD sessions was found. The kinetics of HBV viremia in HD procedures could explain the low viral load which is typically observed in these patients. Further studies to identify the mechanisms responsible for reduction of HBV viremia during HD procedures are under way.

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## Introduction

The frequency of HBV infection is now low in dialysis units in the developed world with prevalence of chronic HBsAg seropositive patients ranging from 0% to 10% in long-term dialysis population [1-2]. Outbreaks of HBV infection, however, continue to be reported in dialysis units with some cases of severe acute hepatitis and even death [3-4]. The epidemiology of HBV among dialysis patients in the less-developed world is not well known; there are scattered reports concerning typically single-center surveys with rates of chronic HBsAg seropositivity ranging between 2% and 20% [5-9].

Only limited information exists on the natural history of HBV infection in patients with chronic kidney disease (CKD) including dialysis patients [10-14]. An accurate assessment of the natural history of HBV in dialysis patients is difficult to obtain as HBV is usually asymptomatic with an apparent indolent course. The disease course of HBV extends over decades rather than years, whereas CKD patients have higher morbidity and mortality rates than those of the general population due to age and comorbidity conditions, making the long-term consequences of HBV infection difficult to establish. Patients with advanced CKD have evidence of immune compromise at both B- and T-cell level. The disease course of hepatitis B in patients on maintenance dialysis is indolent and the reduced immune competence of dialysis patients has been suggested as a possible explanation for decreased necroinflammatory response to HBV infection in these patients [15]. Also, the virological features of HBV among patients on regular dialysis could play a role- it appears that the hepatitis B viral load in serum is rather low and haemodialysis sessions reduce HBV DNA titers in these patients [16-21]. Various mechanisms have been cited including clearance of HBV viremia during hemodialysis sessions [22-23] or peritoneal dialysis treatment [24]. The intradialytic production of interferon (IFN) [25] or other protective substances [26] has been advocated for HCV-infected patients on intermittent dialysis.

The aim of this prospective study was to evaluate if HBV viral load declines during hemodialysis procedures; a highly sensitive assay for HBV DNA quantitation being used. The role of intradialytic kinetics of interferon- $\alpha$  in the drop of HBV DNA during HD sessions was also addressed.

## Patients and Methods

### *Study Group*

All chronic HBsAg carriers undergoing maintenance haemodialysis in seven units in Italy were prospectively included. The patients ( $n=24$ ) underwent long-term haemodialysis (3- or 4-hour treatments; three times weekly), and signed an informed consent; the study protocol was approved by the local ethic review boards. The patients included in the study fulfilled the following criteria; (1) absence of anti-human immunodeficiency virus antibody, (2) exclusion of other causes of chronic liver disease (alcohol, hepatotoxic drugs, autoimmune chronic hepatitis, hemochromatosis, Wilson disease, and alpha-antitrypsin deficiency), and (3) absence of antiviral treatment including  $\alpha$ -interferon based therapy.

### *Methods*

All plasma samples were promptly stored at  $-70^{\circ}\text{C}$  within four hours of sample collection in aliquots until tested by the Abbott RealTime PCR assay; each sample was thawed only for testing. HBsAg was assayed by radioimmunoassay using commercially available kits (Abbott Laboratories). Serum samples were tested for HBV e antigen (HBeAg) and the respective antibody (HBeAb) by radioimmunoassay using available tests (Abbott Laboratories). Serum aspartate aminotransferase (AST; also known as serum glutamic oxaloacetic transaminase) and alanine aminotransferase (ALT; also known as serum pyruvic oxaloacetic transaminase) levels were measured using standard automated analyzers. Upper limits in both the AST (SGOT) and ALT (SGPT) assays were 45 IU/L. All patients were screened by third-generation enzyme-linked immunosorbent assay (anti-HCV 3.0 ELISA; Ortho Diagnostic Systems, Raritan, NY) that detects antibodies (immunoglobulin G [IgG]) to proteins derived from three distinct regions of the hepatitis C virus (HCV) genome (c22-3, c200).

All tests were performed and interpreted strictly in accordance with the manufacturers' instructions. We recorded the pre- and post-dialysis body weight of the HD sessions evaluated in the study.

#### *Virology*

Hepatitis B virus DNA quantitation was performed by a new real-time PCR (Abbott RealTime). The Abbott RealTime HBV test (Abbott Laboratories, USA) is an *in vitro* real-time polymerase chain reaction (PCR) assay for the quantification of HBV DNA in human plasma from HBV-infected individuals [27]. The target region is in the *N* terminal third of the surface gene ensuring that the assay is not impacted by YMDD mutants, HBsAg escape mutants, or drug resistant mutants, as this region is essential for the assembly and secretion of subviral particles, and tolerates only minor structural changes. The Abbott RealTime HBV performances are as follows: Sensitivity, 10 IU/mL; Linear Range, 10 IU/mL (1.0 log IU/mL) to 1 billion IU/mL (9.0 log IU/mL); Inter-Assay Standard Deviation  $\leq$  0.25 log IU/mL; Specificity, 100%; Genotype Detection, accurate quantification of genotypes A, B, C, D, E, F, G, H; Specimen type, serum and plasma; Standardization, World Health Organization (WHO) international standard for hepatitis B virus DNA; Internal Control: added to lysis buffer during extraction and detected to all levels [28].

At the end of the longer (72 hours) interdialytic period, each patient underwent a 4-hour dialysis session. Blood samples were collected from a peripheral vein immediately before the beginning (dialyser empty) and the end of dialysis for determination of HBV DNA levels. Additional blood samples were collected during the subsequent dialysis sessions (mid-week and end-week, respectively)- immediately before and after the dialysis session.

A subset ( $n=12$ ) of patients was tested at baseline for detection of precore and core mutations. Detection of gene promoter polymorphism in nucleotides 1762 and 1764 (basal core promoter, BCP) and nucleotides 1896 (HBV precore) was performed by Inno-Lipa HBV Precore Assay (Innogenetics; Ghent, Belgium).

#### *Interferon measurements*

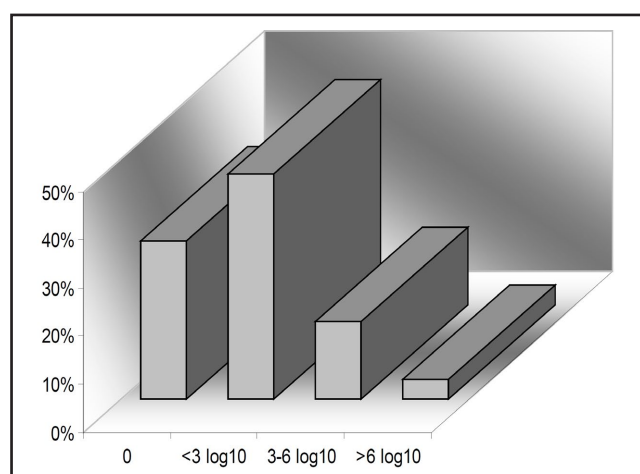
In a subset of HD sessions ( $n=40$ ), we measured recombinant human interferon -alpha (IFN- $\alpha$ ) in serum. Twenty patients were analyzed (two hemodialysis sessions for each patient). We used an enzyme-linked immunosorbent assay for detection of IFN- $\alpha$  in serum (Human IFN- $\alpha$  Instant ELISA). The molecular weight of the recombinant human IFN- $\alpha$  is about 19 kDa consisting of 166 (165 for IFN- $\alpha$ 2a) amino acid residues lacking any N-glycosylation ( $\alpha$ 14 has N-glycosylation). The IFN- $\alpha$  product is manufactured from Pestka Biomedical Laboratories, Inc. (PBL InterferonSource, Piscataway, NJ, US) solely for research use. Samples were not run within 24 hours but were stored frozen at  $-20^{\circ}\text{C}$  to avoid loss of bioactive human IFN- $\alpha$ ; repeated freeze-thaw cycles were not made. Semiquantitative measurement of human IFN- $\alpha$  was performed.

#### *Statistical analysis*

Serum HBV DNA values and aminotransferase levels were logarithmically ( $L_{10}$ ) transformed to obtain normal distribution and then subjected to statistical tests. The Shapiro-Wilk test was used to assess that the samples came from a normally distributed population. Serum HBV DNA values by Abbott RealTime Assay and aminotransferase values are expressed as mean $\pm$ SD or median values with respective range or 95% confidence intervals (CIs). Means between two groups were compared using Student's *t*-test. Student's *t*-test (paired data) was used to compare serum HBV DNA levels at the beginning *versus* the end of hemodialysis (HD) sessions. Categorical data were compared by chi-squared test with Yates' correction for continuity. All *P* are two sided, and *P* less than 0.05 was considered significant. Simple linear regression analysis was used to analyse the relationship between mean HBV DNA levels before dialysis and the percentage reduction of HBV DNA after HD session. Statistical analysis was performed using the Free Statistics Software (version 1.1.23-r7), Office for Research Development and Education, URL <http://www.wessa.net/> by Wessa P. (2012).

## **Results**

The demographic data of the study patients are shown in Table 1. Fourteen patients underwent chronic HD using polysulphone membrane, four by polymethylmetacrylate,



**Fig. 1.** Distribution of HBV viral load ( $\log_{10}$  IU/mL) among HBsAg positive patients ( $n=24$ , 70 haemodialysis sessions) before HD.

**Table 1.** Characteristics of patients included in the study group ( $n=24$ )

Characteristics	( $n=24$ )
Age, years	59.9±14.7
Time on dialysis, months	127.6±102
Gender, female (%)	5 (20.8%)
Race, Caucasian	23 (96%)
Creatinine, mg/dL	9.4±3.6
SGOT, UI/L	25.7±26.1
SGPT, UI/L	26±44.09
GGT, UI/L	63±94
Total protein (g/dL)	7.4±0.7
Albumin (g/dL)	5.7±0.8
Bilirubin (mg/dL)	0.34±0.7

Data presented as mean±standard

three by hemophane, and three by polyacrylonitrile. Data of 70 haemodialysis sessions were collected.

Causes of ESRD were as follows: nephroangiosclerosis ( $n=5$ ), chronic glomerulonephritis ( $n=6$ ), diabetic nephropathy ( $n=2$ ), polycystic kidney disease ( $n=1$ ), chronic pyelonephritis ( $n=4$ ), and ESRD of unknown or other causes ( $n=6$ ). The frequency of HBsAg positive patients with detectable HBV viremia in serum was 20% (20/24); the rate of HBsAg positive/HBeAg positive individuals was 8.3% (2/24). Positive anti-HCV serologic status was reported in two patients (8%).

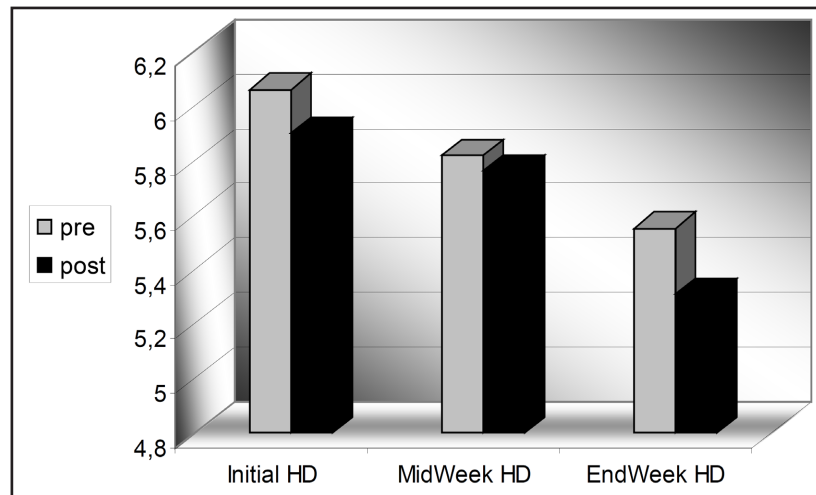
There was a significant drop of HBV viral load at the end of HD session- mean HBV DNA levels at the beginning and the end of HD sessions were  $5.92 \log_{10}$  IU/mL (95% CI, 5.34 to  $6.28 \log_{10}$  IU/mL) vs.  $4.79 \log_{10}$  IU/mL (95% CI, 4.23 to  $6.15 \log_{10}$  IU/mL), respectively ( $P=0.02$ ). The mean HBV DNA levels in the subgroup of HBsAg positive patients with detectable HBV DNA in serum were  $5.96 \log_{10}$  IU/mL (95% CI, 5.32 to  $6.28 \log_{10}$  IU/mL) (before HD sessions). The majority of patients had pre-dialysis HBV DNA titers under  $\log_{10}$  IU/mL (67/70=95.7%) (Figure 1).

HBV DNA reduction occurred in many HD sessions [42% (29/70)], irrespective of dialysis membrane, patient characteristics, or HD session (mid-week, end-week hemodialysis or 72 hours after). Four HBsAg positive HBV DNA negative patients underwent twelve HD procedures without detectable HBV DNA in serum before/after HD session. Identical HBV DNA titers were found in nine patients (9/70=13%); an increase in HBV DNA levels at the end was observed in 20 HD procedures (28%). The mean percentage reduction of HBV viral load at the end of HD session compared with the beginning was around  $26.3\% \pm 12.5\%$ .

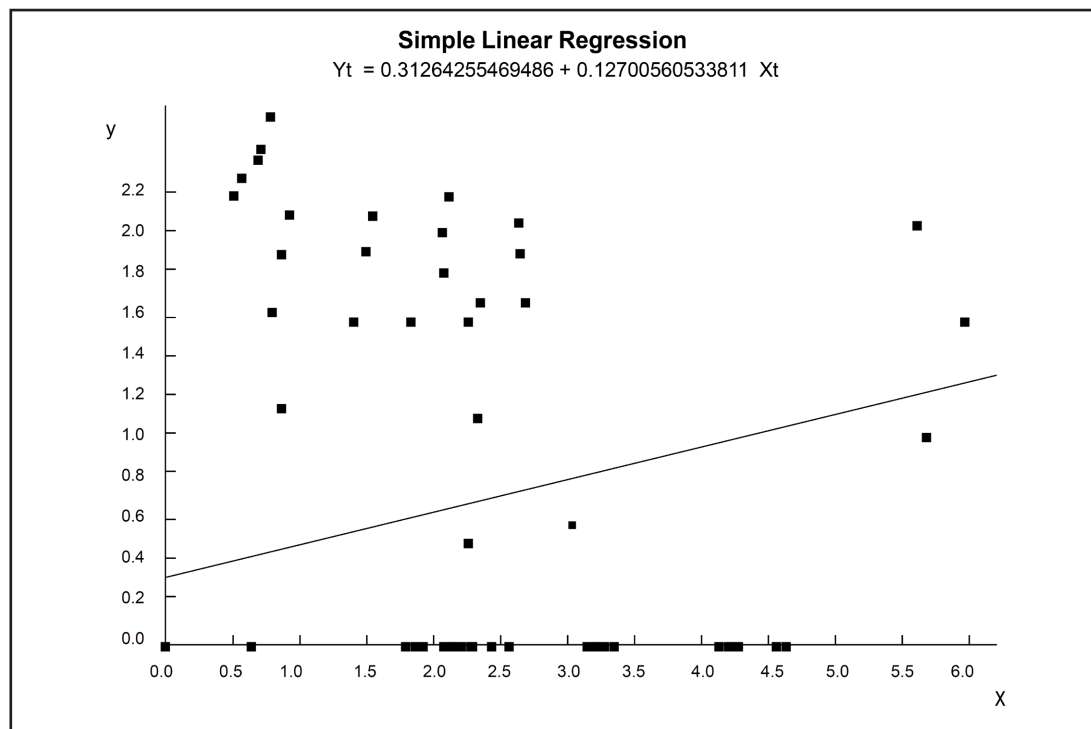
Figure 2 shows mean HBV titers before/after each HD session (mid-week, end-week or 72 hours after, respectively). Mean HBV titers at pre-dialysis phase were higher in the HD session at the beginning of the week than the mid-or end-week procedures, even if no significant differences occurred ( $P=0.62$ ). We found a significant relationship between mean HBV DNA levels at the start of dialysis and percentage drop of HBV DNA during HD sessions occurred [simple linear regression-analysis of variance:  $F$ -test=5.418026, rho (least squares)=0.307] (Figure 3).

A decrease of serum IFN- $\alpha$  levels in 15 (37.5%) HD sessions was noted, 22 (55%) HD procedures did not show modifications of IFN- $\alpha$  concentration. An increase of serum IFN- $\alpha$  levels was found in three (7.5%) HD sessions. We did not observe relationship between HBV DNA reduction during HD procedure and IFN kinetics (data not shown) by the analysis of a subset ( $n=40$ ) of HD sessions. No PC/BCP mutations were detected.

**Fig. 2.** HBV DNA titers ( $\log_{10}$  IU/mL) before/after haemodialysis at each session.



**Fig. 3.** HBV DNA levels and percentage reduction of HBV DNA during HD procedures.



## Discussion

Our knowledge on the consequences related to HBV infection in the dialysis population remains largely incomplete despite important advances in the control of HBV spread within dialysis units [29]. The available information suggests slow progression of HBV-related liver disease in the dialysis population, and various mechanisms have been cited including the virological features of HBV. Only a few and small series of HBsAg positive patients have been evaluated to detect HBV viremia by molecular methods in patients on maintenance dialysis [30-34].

The goal of this study was to assess whether the haemodialysis session itself leads to a reduction in HBV viraemia; to this aim, we have used RealTime PCR technology provided with higher sensitivity than other techniques, as an example bDNA technology, for measuring the viral load of hepatitis B virus (10 IU/mL vs. 2,000 copies/mL, respectively) [27]. Thus, the



frequency of chronic HBsAg carriers on long-term dialysis without detectable HBV viremia in serum was lower than that observed in other surveys, 17% vs. 50% [18]. HBsAg positive patients with negative results by RealTime PCR technique at the beginning of the study were enrolled as prior evidence showed changes in HBV DNA titers over time with intermittent positive HBV DNA testing [17].

We found that the HD procedure *per se* leads to a reduction in HBV viral load; this phenomenon occurred irrespective of patient (or dialysis) characteristics or type of dialysis membranes. Our results are in keeping with information coming from other sources concerning HBV [18-20] or HCV [35]. The mechanisms underlying the intradialytic drop in HBV viraemia remain to be clarified. One of the factors for low HBV viral load prior to hemodialysis could be hemodilution as recently observed for alanine aminotransferase levels [36]; however, the data reported in Figure 2 are not in keeping with this. In fact, higher levels of HBV DNA at pre-dialysis phase were found in the first dialysis of the week where the hemodilution effect is much greater than at the midweek or end-of week sessions. The viral half-life that has been calculated for HBV (2-3 days) [37] could play a role in explaining the kinetics of HBV viral load shown in Figure 2.

Some authors described HBV DNA in the dialysate [22-23]; the passage of HBV DNA across the membrane of the dialyzer filter into the spent dialysate was suggested even if the estimated size of HBV particles (42 nm) does not allow the escape of intact virus through the dialysis membrane into the dialysate. Adsorption of the virus onto the haemodialysis membrane and pressure-dependent destruction could be major potential mechanisms of virus clearance during the haemodialysis procedure, as in HCV [38-39]. The synthesis of various cytokines [40-42] or the removal of factors required for viral production have been also cited to explain the intermittent reduction of HBV viraemia in patients on long-term HD.

Theoretically, the lowered HBV DNA levels at the end of the haemodialysis procedure may be caused by the translocation of the virus into the extravascular compartment during the HD sessions. The shift of body fluids containing HBV virions from the extravascular to the intravascular compartments over the interdialytic period is in keeping with the increase of the HBV load at the beginning of the next HD session, as shown in the current study. The post-dialysis rebound of plasma solutes is a relevant drawback of maintenance haemodialysis and is currently an area of active research [43].

The intradialytic changes in IFN- $\alpha$  levels reported in anti-HCV positive patients and thought to be associated with the HCV viral load [25] were not seen in our cohort of HBV-infected patients. We found an increase in IFN- $\alpha$  in a minority of HD procedures only (7.5%); and no relationship between reduction of HBV viral load and intradialytic kinetics of IFN- $\alpha$  occurred. Our investigation on the kinetics of circulating IFN- $\alpha$  in HD patients had preliminary nature- a limited number of HD sessions ( $n=40$ ) was analyzed, and a semiquantitative analysis was made.

Recent information has shown that the viral load in chronic HBsAg-positive carriers on maintenance dialysis is low [17-19] and relatively stable over years despite the immunodeficiency conferred from chronic uraemia [17]. The intermittent reduction of the viral load and the low number of HBsAg positive/HBeAg positive patients, as listed in our study, support this. Four (17%) of our patients were repeatedly negative by RealTime PCR technology. HBsAg positive patients have been recently identified as active carriers in the case of a viral load of  $\geq 20,000$  IU/mL in HBeAg positive and 2,000 IU/mL in HBeAg negative patients [44].

Our study shows some potential shortcomings. First, we have used RealTime PCR for measuring hepatitis B virus DNA in serum despite heparin is commonly used during haemodialysis sessions in developed countries. It has been suggested that heparin is an unsuitable anticoagulant for plasma DNA analysis because it could inhibit PCR; however, recent data does not confirm this [45]. Second, we noted no impact of various dialysis membranes on the intradialytic reduction of HBV DNA during haemodialysis. Some authors [46] found differences in the kinetics of viral load according to the type of membrane used in HCV positive dialysis patients (i.e., synthetic versus cellulosic membranes). The majority

of our patients used high-biocompatibility dialysis filters as low-biocompatibility membranes (i.e., cuprophane) are surpassed in developed world; also, the limited number of patients hampered an appropriate comparison. Third, we have not corrected our results on HBV DNA kinetics by fluid loss during dialysis, as performed by others [36]. In fact, when water is extracted during haemodialysis, the plasma becomes concentrated and content in plasma shows higher concentrations reflected in haematocrit increases. The passage of IFN- $\alpha$  or hepatitis B virus into the dialysate compartment has not been expected due to the size of the respective molecules. Applying the correction of HBV DNA kinetics by haematocrit increases [21], decreases in HBV DNA viral load should become more pronounced, while increases smaller or disappeared.

We detected no PC/BCP mutations by Line Probe Assay technique. The low HBV viral load in our series could explain this- in fact, recent data suggest a significant relationship between high HBV viral load and frequency of PC/BCP mutations in chronic hepatitis B [47]. We tested a small number of patients and this precludes more definitive conclusions.

## Conclusion

We observed that the hemodialysis procedure *per se* lowers HBV DNA levels and the intradialytic kinetics of IFN- $\alpha$  is not apparently responsible of this phenomenon. Although other mechanisms cannot be excluded, we suggest that the intermittent reduction of HBV viremia in patients on long-term haemodialysis is implicated in the mild course of hepatitis B-related liver disease in this population.

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